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AN ECOFRIENDLY APPROACH OF LIPASE PRODUCTION BY MARINE ACTINOMYCETES USING AGRO WASTE (NEEM CAKE)

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ABSTRACT

A Gram positive, sporulative, thermophilic marine Actinomycetes strain capable of producing extracellular lipase was isolated from marine soil, collected from 4-5 feet depth of the coastal area of kanyakumari district, in Tamilnadu. The efficiency of neem cake as a substrate for lipase production by marine Actinomycetes strain in solid state fermentation (SSF) has been studied and reported. The Neem oil cake is rich in protein, cheap, abundantly available being an agro industrial waste and can be constructively used as one of the main constituent in medium formulation. The growth conditions were optimized for the maximum production of enzymes. Parameters such as pH, temperature, carbon and nitrogen sources were optimized. The lipase activity was found to be maximum in solid state fermentation in alkaline conditions at pH 9.0 under thermophilic conditions of temperature 47ºC.

KEY WORDS: **Neem cake, Marine Actinomycetes, Lipase, Agro waste.**

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INTRODUCTION

Man has been using enzymes for ages, in different forms, as extracts obtained from vegetables or animal organs or as microbes[1]. A large number of enzymes are being produced and sold for various purposes and the blooming industrial enzyme market is one of the major revenue generators in the life sciences industry sector[2]. The demand for the industrial enzymes, particularly of microbial origin is greatly increased due to their application in a wide range of processes. Microbial enzymes are often more useful than enzymes derived from plant or animals because of their varieties in catalytic activities, high yield, easy genetic manipulation and rapid growth of microbes[3]. Lipases have gained special attention over a few decades owing to their ability to act in micro-aqueous environment, and catalyze esterification, transesterification, aminolysis, and acidolysis reactions[4]. Microbial lipases are produced mostly by submerged culture, but solid state fermentation methods can also be used[5]. A solid state fermentation is a high recovery method for the production of industrial enzymes. They provide much advantage over submerged fermentation for production of the enzyme lipase India is an agricultural country, in which large amount of agro wastes are produced. Marine actinomycetes are considered to be potential microorganisms capable of producing novel secondary metabolites. The present investigation aims to study the lipase activity of actinomycetes isolated from the sediments[6].

MATERIALS AND METHODS

The marine sediment samples were collected from 4-5 feet depth of the coastal area of kanyakumari district (8.0780° N, 77.5410° E) ,Tamilnadu, India. The samples were immediately transferred to squeezed glass bottles and stored at 4 ^{o}C. The neem cake was purchased from local markets in Coimbatore, Tamilnadu, India and they are packed in polythene bags and brought to the laboratory. The chemicals were purchased from Himedia, Mumbai.

(i) Screening of marine actinomycetes

One gram of the marine sediment sample was taken and mixed with 100 ml of sterile distilled water and kept in a shaker (SI 300/300R) for 10 minutes and the supernatant was serially diluted. The actinomycetes isolation agar media were sterilized. After solidification 0.5 ml of the sample was aseptically transferred to the sterile petriplates[7]. They were stored at 37 ^{o}C for 10 days and examined for colony formation. The actinomycetes strains were purified by multiple streaking techniques used for lipase production.

(ii) Screening and identification of lipase producing actinomycetes

The tributryin agar plates were prepared and the test organisms were streaked on the agar surface. The plates were incubated for 5 days at 37 ^{o}C. After the incubation period, the lipase producing actinomycetes were screened by zone formation. The higher zone forming actinomycetes were selected for production of lipase[8]. 10g of desired neem cake was mixed separately with nutrient media and sterilized. The positive strain actinomycete was inoculated into the respective waste media. The flasks were kept in shaker (SI 300/300R) at 180 rpm and incubated for seven days at 37 ^{o}C[9]. The supernatant from the culture broth was collected by centrifugation at 6000 rpm for 15 minutes at 4 ^{o}C. The supernatant was used as a crude enzyme source to estimate lipase production. The enzyme extract was partially purified by ammonium sulphate fractionation (70% saturation) and dialysis was done for further applications[10].

(iii) Optimization of different parameters for lipase production

Sterile production medium was prepared under different parameters such as p^{H}, temperature, carbon sources and nitrogen sources. Each flask was incubated at different p^{H} (5,6,7,8, & 9), and temperature (30 ^{o}C, 37 ^{o}C & 47 ^{o}C) , carbon sources (fructose, sucrose, and

dextrose) and nitrogen sources (urea, ammonium nitrate and peptone). They were similar to earlier reports[11,12,13,14,17].

RESULTS AND DISCUSSION

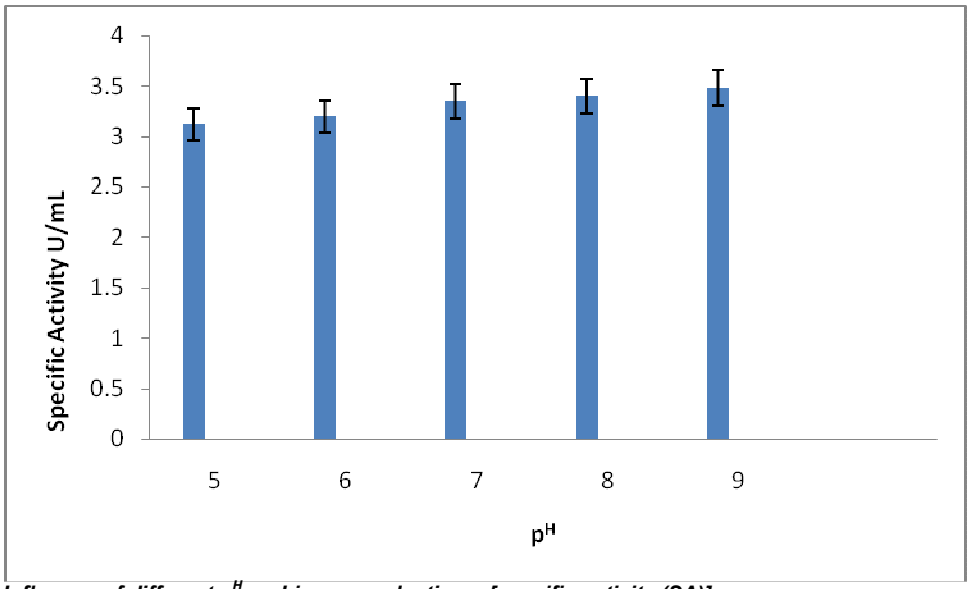
The positive actinomycetes strain was confirmed by the production of clear zones around the colonies in tributryin agar plates. Among 8 lipase producing actinomycetes Mac - 5 (Figure -1) showed the highest zone formation than the other microbes and it was selected as the best strain for lipase production. An actinomycetes strain was identified by powdered white, mycelia formation and was confirmed as rod shaped gram positive bacteria. The thermal stability of the actinomycetes was checked and recorded at 60-80 ^{o}C. Marine actinomycetes are highly thermo stable in nature, so they were used for the production of lipase and the enzyme extract was used as a good detergent additive which can withstand higher temperatures. From the results (figure 2-5) it was observed that the optimum lipase production was at p^{H} 9 (3.48 U/ml) (castro et al., 2005). temperature 47^{o}C (3.47 U/ml), carbon source (dextrose) (3.37 U/ml) and nitrogen source (urea) (3.39 U/ml). The above results were similar to earlier reports[15.]

Screening of Marine Actinomycetes (MAC-5)

Figure 1
Highest zone formation than the other microbes and it was selected as the best strain for lipase production

1. Influence of p^H

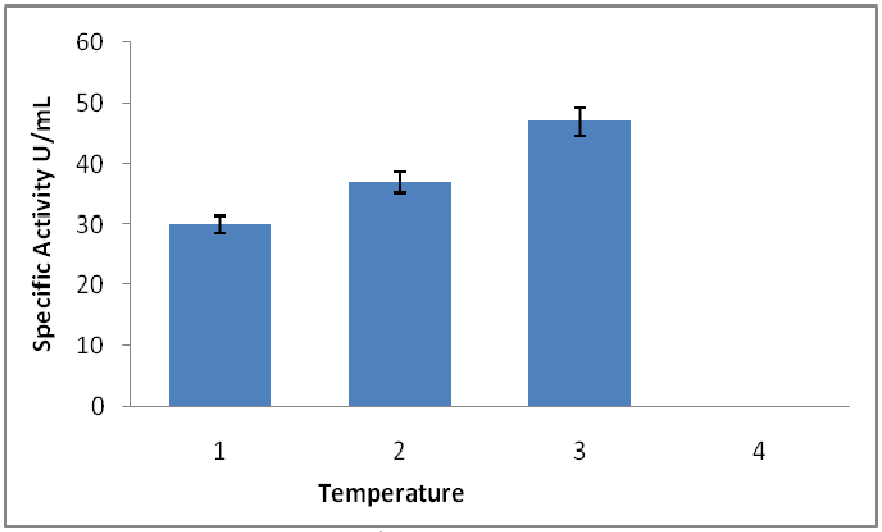
Graph 1
Influence of different p^H



Influence of different p^H on Lipase production – [specific activity (SA)]

2. Influence of Temperature

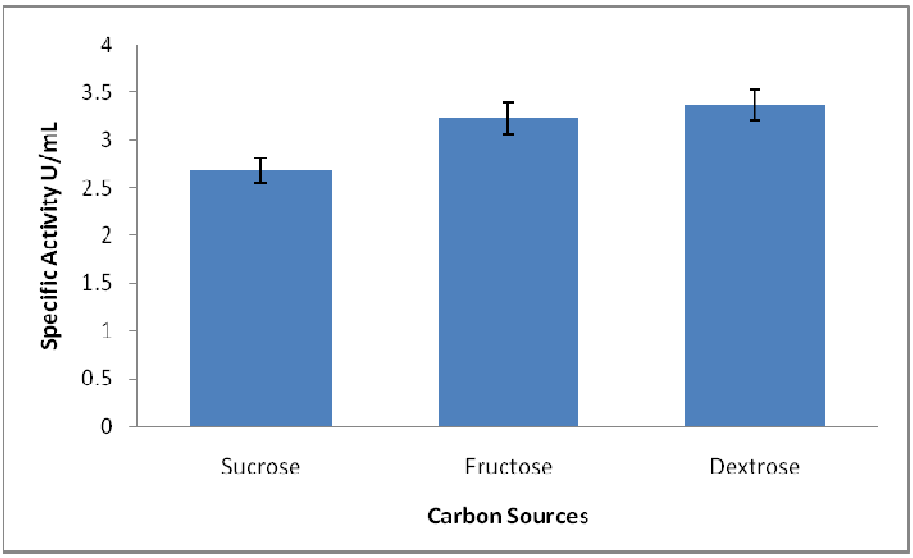
Graph 2
Influence of different Temperature ^oC



Influence of different Temperature ^oC on lipase production – [specific activity(SA)]

3. Influences of Carbon sources

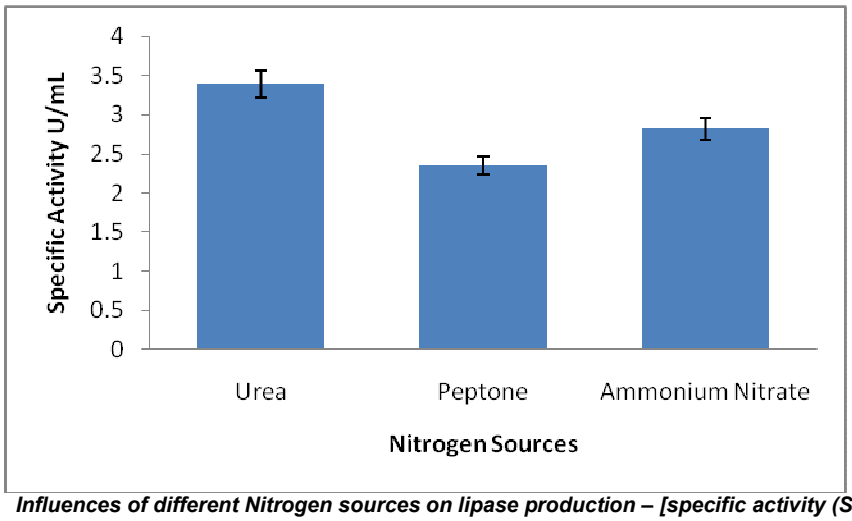
Graph 3
Influences of different Carbon sources



Influences of different Carbon sources on lipase production – [specific activity (SA)]

4. Influences of Nitrogen sources

Graph 4
Influences of different Nitrogen sources



Influences of different Nitrogen sources on lipase production – [specific activity (SA)]

CONCLUSION

India is an agricultural country. The utilization of agro industrial wastes for the production of enzymes as substrate showed an eco friendly management of wastes, they by reducing the effects of pollution in the environment. So it was concluded that the agro industrial waste can be better utilized for the production of novel enzymes like lipase by using marine thermophilic actinomycetes.

REFERENCES

1. Benjamin S, Pandey, A. Mixed solid substrate fermentation: a novel processes for enhanced lipase production by *Candida rugosa* . Act a Biotechnological, 18: 315 - 324, (1998).
2. Brockman H.L. General features of lipases: reaction scheme, interfacial structure and experimental approaches in lipases. B. Borg storm and H.L. Brockman Amsterdam (Eds.), Elservier,pp. 3 - 46,1984.
3. Boekema AB, Breuer M, Hauer B, Koster M, Rosenau F, Jaeger KE and Tommassen J,. Hexadecane and tween 80 stimulated lipase production in *Burkholderia glumae* by different mechanisms. Appl Env microbial, (7): 3838 - 3844, 2007.
4. Joseph B, Ramteke PW, Kumar PA. Studies on the enhanced production of extracellular lipase by *Staphylococcus epidermidis*. J Gen Appl Microbiol, 52: 315 - 320, 2006.
5. Chisti Y. Solid Substrate Fermentations, Enzyme Production, Food Enrichment. In: Flickinger M.C., and S.W. Drew (Eds.), Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation, Wiley, New York,pp.2446 - 2462,1999.
6. Christen P, Angeles NM, Corzo G, Fares A and Revah S. Microbial Lipase Production on a polymeric resin. Biotechnol Technol, (9): 597-614, 1995.
7. Rathi P, Goswami VK, Sahai V and Gupta R. Stastical medium optimization and production of a thermo stable lipase from *Burkholderia Cepapcia* in a bioreactor. J App Microbiol, (93): 930 - 945, 2002.
8. Nahas E. Control of lipase production by *Rhizopus oligosporus* under various growth conditions. Gen Microbial, (27): 227 - 238 2000.
9. Ramachandran S, Singh SK, Larroche C, Soccol CR, and Pande, A. Oil cakes and their biotechnological applications. Biores Technol, (98): 2000 - 2009, 2006.
10. Sangeetha R, Geetha A, and Arulpandi I. Production of Protease and lipase by Bacillus Licheniformis VSGI: Production, purification and characterization. Brazilian J Microbial, (41): 179 - 185 2010.
11. Kamini NR, Mala JGS, and Puvankrishnan. Lipase production from Aspergillus niger by solid state fermentation using gingely oil cake. Process Biochem, (33): 505 - 511, 1998.
12. Castro LR, Polato CMS, Sant Anna Jr GL and Freire DMG. Economic analysis of lipase production by *Penicillium restrichum* in solid state and submerged fermentations . Biochem Eng J, (4): 239 - 247, 2000.
13. Li H and Zhang X. Characterization of thermostable lipase from themophilic Geobacillus species. Twl Prot Express Purific, (42): 153 - 159, 2005.
14. Mahler GF, Kok RG, Cordenons A, Hellingner K.J and Nudel BC. Effects of carbon sources on extracellular lipase production and lipA transcription in *Acinetobacter calcoaeticus*. J indus Microbio and Biotech, (24): 25 - 3, 2000.
15. Moreira KA, Albuquerque BF, Teixeira MFS, Porto ALF, Filho Lima JL. Application of proteases from Nocardiopsis sp. as a laundry detergent additive. W J Microbio and Biotech, (18): 307 - 312, 2002.
16. Sharma R, Christi Y, and Banerjee UC. Production purification characterization and application of lipases. Biotech Advances, (19): 627 - 662, 2001.
17. Tumane PM, Meshram VG and Wasnik DD. Comparative study of antibacterial activity of peel extracts of *Citrus aurantium* L. (bitter orange) and C*itrus medica* L. (lemon) against clinical isolates from wound infection. Int J Pharm Bio Sci 5(1): 382 - 387, 2014.